

**MONOAMINES IN THE BRAIN OF TARANTULAS
(*APHONOPELMA HENTZI*) (ARANEAE, THERAPHOSIDAE):
DIFFERENCES ASSOCIATED WITH MALE
AGONISTIC INTERACTIONS**

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ABSTRACT. Experiments were conducted to determine the effects of male-male agonistic encounters on changes in monoamine neurotransmitter concentrations in the supraesophageal ganglion (brain) of the tarantula, *Aphonopelma hentzi*. Serotonin levels were significantly reduced 30 min after fighting in both dominant (66.5 ± 9.1 SE nmol/mg protein) and subordinate (42.8 ± 7.6) animals as compared to isolated controls (89.7 ± 13.2), and these differences persisted for up to 24 h. A similar decrease was found for octopamine concentrations in dominant (43.7 ± 7.7) and subordinate (31.2 ± 4.9) spiders when compared to controls (56.9 ± 5.8). In addition, serotonin and octopamine levels were significantly lower in subordinate vs. dominant spiders. Agonistic interactions had no effect on the concentrations of dopamine, norepinephrine, and epinephrine. In isolated control spiders, serotonin (89.7 ± 13.2 SE nmol/mg protein) was present in highest concentration in the brain, followed by octopamine (56.9 ± 5.8 nmol/mg), dopamine (22.4 ± 3.8 pmol/mg), norepinephrine (15.3 ± 4.7 pmol/mg), and epinephrine (0.57 ± 0.2 pmol/mg). The results indicate that following agonistic encounters, monoamine concentrations in the brain decrease to different levels in winners and losers. This is the first demonstration that the establishment of social status causes changes in brain monoamines in spiders.

Keywords: Agonistic interactions, *Aphonopelma*, CNS monoamines, males

Activation of monoaminergic systems in the central nervous system (CNS) has been implicated in the mediation of short-term and chronic physiological stress responses as well as aggressive and social dominance relationships in numerous taxa (Eichelman 1987; Bicker & Menzel 1989; Haney et al. 1990; Summers et al. 1995). Most of this work has focused on social interactions in vertebrates exposed to staged encounters between conspecifics under laboratory conditions. For example, the social status of subordinate animals is associated with an increase in the utilization of the indolalkylamine neurotransmitter (NT) serotonin (5-HT, 5-hydroxytryptamine) in various brain regions from fish to mammals (Haney et al. 1990; Winberg et al. 1997; Punzo 2000a). In addition, activation of brain catecholaminergic systems including dopamine (DA), norepinephrine (NE), and epinephrine (Epi) in vertebrates is associated with increased levels of aggression and dominance (Eichelman 1987; Matter et al. 1998).

In general, the brain or supraesophageal

ganglion (SEG) of arthropods lies above the esophagus and consists of three major brain regions: the protocerebrum, which contains the 'higher brain centers' including the optic lobes and nerves; the central body, corpora pedunculata and a variable number of ganglia and associated neuropils; the deutocerebrum, which innervates the antennae (reduced in arachnids); and the tritocerebrum, which innervates the mouthparts (Horridge 1965; Gupta 1987). In spiders, the CNS is highly condensed anteriorly, and the SEG consists primarily of the cheliceral and optic nerves, optic neuropils (masses of axonal fiber tracts) for primary and secondary eyes ('corpora pedunculata'), and a loosely organized central body (Babu 1985; Wegerhoff & Breidbach 1995). There have been few studies on neurochemical parameters associated with behavior in general, and agonistic interactions specifically, in arthropods and other invertebrates. For example, increases in protocerebral RNA and protein synthesis have been shown to accompany learning in molluscs (Kerkut et al.

1970; Adamo & Chase 1991), decapod crustaceans (Punzo 1985), insects (Lin & Roelofs 1992; Punzo 1996), and spiders (Punzo 1988a). Cycloheximide-induced inhibition of brain protein synthesis impaired learning and memory in insects (Jaffe 1980) and spiders (Punzo 1988a), as well as innate phototactic behavior in tenebrionid and passalid beetles (Punzo & Jellies 1980). Changes in levels of brain monoamines have been implicated in a variety of ontogenetic shifts in behavior in honeybee workers including the onset of nest-guarding behavior (Moore et al. 1987) and discrimination between olfactory cues (Macmillan & Mercer 1987).

With respect to aggression and agonistic interactions, octopamine turnover rates increased significantly in crickets after fighting with conspecifics (Adamo et al. 1995). Increased foraging activities and nest defense were correlated with higher concentrations of octopamine (OA), dopamine (DA), and serotonin (5-HT) in the SEG of worker honeybees (Harris & Woodring 1992). Indeed, it has been suggested that OA is part of a general arousal system which prepares insects for a variety of vigorous skeletal-muscular activities, territorial defense, and helps the animal deal with stressful conditions (Corbet 1991; Orchard et al. 1993). Increased 5-HT levels in the brain (SEG) have been implicated in the onset of flight behavior in weevils (Guerra et al. 1991). Lobsters exhibiting dominance over conspecifics exhibited higher levels of CNS 5-HT when compared with subordinate animals (Kravitz 1988).

Changes in SEG amine concentrations as well as other NTs were shown to be associated with ontogenetic shifts in behavior in solifugids (Punzo 1993, 1994). First nymphal instars (N1) typically have poorly developed chelicerae, and are gregarious, do not hunt prey, and remain in the nest with their siblings and maternal parent. However, after molting, second-instar nymphs (N2) possess functional chelicerae and become aggressive (Punzo 1998a). They will cannibalize one another if they do not disperse from the nest. This pronounced increase in aggression is associated with significant changes in brain 5-HT and DA levels, although OA levels remained relatively constant throughout postembryonic development (Punzo 1994). In addition, later nymphal instars (N5—N8) exhibited higher brain concen-

trations of acetylcholine (ACh), norepinephrine (NE), and acetylcholinesterase (AChE) as compared to younger instars (Punzo 1993).

Theraphosid spiders exhibit a variety of aggressive behaviors. Some of these involve male-male agonistic interactions (Baerg 1958; Minch 1977; Punzo & Henderson 1999; Punzo 2000b), while others involve males and females, especially during courtship and mating (Costa & Perez-Miles 1992; Shillington & Verrell 1997), and sometimes end in sexual cannibalism (Punzo & Henderson 1999). A previous study showed that agonistic interactions between paired conspecific males of *Aphonopelma hentzi* (Girard 1854) were observed in 24 out of 27 (88.9%) staged encounters in the laboratory (Punzo & Henderson 1999). These encounters were initiated by vigorous leg-fencing with each protagonist pushing forcefully against its opponent. These fencing bouts were interrupted from time to time with at least one of the males exhibiting a threat display (elevation of the anterior end of the body, first pair of legs and pedipalps, and opening of the fangs). At least one male exhibited a strike response toward his opponent in 11 out of 24 cases (45.8%). In eight of these instances (33.3%), one of the males was killed.

The purpose of this study was to investigate neurochemical parameters associated with agonistic interactions between males of the theraphosid spider, *Aphonopelma hentzi*. Neurotransmitters and comodulators are important regulatory molecules required for the transmission of information (nerve impulses) along neural pathways involved in the control of motor movements as well as 'mood' and motivational states (Ansell & Bradley 1973). Specifically, we were interested in whether or not there were any differences in the concentrations of monoamines (OA, DA, NE, Epi, 5-HT) in the brains (SEG) of dominant ('winners') vs. subordinate ('losers') males following agonistic encounters. To our knowledge, this is the first study to address neurochemical correlates of aggression in spiders.

METHODS

Animals.—Males were collected during July and August of 1997 at a site 3.5 km S of Elgin, Texas (30°32'N, 97°29'W; Bastrop County). This site consisted of a dry wash and surrounding flood plain consisting of sand,

gravel and adobe soils, with numerous rocks, rock crevices, and burrows. The dominant vegetation included prickly pear cactus (*Opuntia*), catclaw (*Mimosa*), mesquite (*Prosopis*), broom weed (*Xanthocephalum*), and mesquite grass (*Bouteloua*). Adult males ranging in size from 4.2–6.7 g were abundant during this period and were easily found moving about the surface between 2000–0300 h (Central Standard Time). Spiders were collected and weighed to the nearest 0.1 g using a Ohaus Model 87 portable electronic balance.

Spiders were transported to the laboratory and housed individually in plastic cages (20 × 16 × 8 cm). They were provided with water *ad libitum* and fed three times per week to satiation on a mixed diet of crickets (*Gryllus* sp.), mealworms (*Tenebrio molitor*), and grasshoppers (*Schistocerca* sp.). They were maintained at 22 °C ± 1°, 65% RH, and a photoperid regime of 12L:12D in a Percival Model 805 environmental chamber (Boone, Iowa). Adult males were kept in these conditions for two weeks and then re-weighed on the day before the initiation of encounter trials. Since previous studies on arachnids have indicated that differences in body size can influence the outcome of aggressive encounters (Faber & Bayliss 1993; Punzo 1998c, 2000b), only males of approximately similar size (6.2–6.7 g) were used for encounter trials and subsequent neurochemical analyses. Voucher specimens have been deposited in the Invertebrate Collection at the University of Tampa.

Encounter trials.—We used a rectilinear glass arena (26 × 16 × 12 cm) divided into halves by an opaque divider to stage conspecific male encounters as described by Punzo (1998c). To summarize, the floor of the arena was provided with a layer of loose sand to a depth of 2 cm. All observations were conducted under Black lighting (BioQuip Onc., Model 2804, Gardena, California). We used a Panasonic PS 150 tape recorder to record verbal descriptions of each encounter.

Before each encounter trial, a male spider (chosen at random) was placed at each end of the arena, separated by the opaque divider. A trial was initiated by removing the divider and allowing the animals to interact. Within a period of time ranging from 0.5–8.5 min over all trials, the contestants made contact with one another (usually with a front leg). In a few trials, one of the spiders would immediately

attempt to flee after making initial contact with its opponent. These trials were not used in data analysis. In all other cases ($n = 60$ trials), following initial contact, one of the spiders would begin to push with its front pair of legs against its opponent. The other spider rapidly responded in a similar fashion (leg-fencing). In other cases, after initial contact, one or both spiders would exhibit the threat display, followed by another bout of leg-fencing. In a few instances the fighting escalated until one or both spiders attempted to bite the other. An encounter trial was terminated if at any time during the encounter one of the spiders backed away and rapidly fled from the vicinity of the other spider and attempted to crawl out of the chamber. The spider that held its ground was recorded as the 'winner' (dominant animal), and the spider that fled, the 'loser' (subordinate). Each pair of contestants were subjected to only one encounter trial as described by Summers & Greenberg (1995) in their study of male-male aggression in lizards. We conducted a total of 60 encounter trials comprising 60 pairs of contestants ($n = 120$).

Neurochemical analyses of brain tissues.—Immediately following their designation as dominant or subordinate (based on the outcome of encounter bouts), paired contestants were randomly assigned to one of three groups; each group consisted of 40 spiders (20 pairs). Spiders in group 1 (G1) were anaesthetized with CO₂ thirty min after encounter trials, and their brains (SEG) removed in a cold room, weighed to the nearest 0.1 g on an electronic analytical balance, and frozen at –80°C as described by Punzo (1988b) for subsequent neurochemical analyses. Group 2 (G2) and group 3 (G3) spiders were anaesthetized and their brains frozen at 24 hr and 48 h, respectively, after encounter trials. In this way, we were not only able to determine what neurochemical changes, if any, followed male-male aggression, but also how rapid the response might be, and how long these changes might persist. The brains from another group of 20 spiders (G4) maintained in isolation and not exposed to encounter trials were used as controls.

After thawing, all glandular and peripheral fatty tissue was carefully removed from the surface of the SEG (Murdock & Omar 1981). The SEG were then weighed to the nearest 0.01 g on a Sartorius Model 54C electronic

analytical balance. Brain protein determinations were conducted using the standard procedure described by Lowry et al. (1951) and expressed as percent (brain protein/brain weight) (Meyer et al. 1984). The SEG from the dominant and subordinate spiders were analyzed to determine the concentrations of the monoamine neurotransmitters, 5-HT, OA, DA, and NE, using high performance liquid chromatography with electrical detection (HPLC-ED, Beckman Model 47A) as described by Brandes et al. (1990). To summarize, each brain tissue sample was placed in a 750 μ l glass vial and homogenized in 50 μ l of a 200 mM perchloric acid (PA) solution. Following homogenization, an additional 50 μ l of PA were added to each vial. Samples were then centrifuged at 10,000 g and 4 °C for 3 min in a Sorvall Model 100A high speed refrigerated centrifuge. Twenty μ l of supernatant were injected directly into the HPLC column (40 cm in length, with a 0.2 μ pore diameter) packed with Hypersil and provided with a Hewlett-Packard 760E detector (0.40 V). The mobile phase (flow rate, 3000 psi) used to elute the monoamines consisted of 12% acetonitrile, 20 mM sodium acetate, 100 mM sodium dihydrogen orthophosphate, 2.5 mM octane sulfonic acid, and 0.3 mM EDTA disodium salt adjusted to pH 4.2 and filtered through a 0.45 μ m filter. Each sample was compared to 5-HT and DA standards tested at the beginning of each assay run and retested at 30 min intervals. Monoamine concentrations were expressed as nmol or pmol/mg protein as described by Meyer et al. (1984).

All statistical procedures followed those described by Sokal & Rohlf (1995). Comparisons between mean concentrations of monoamine NTs for the various groups were conducted using an analysis of variance (ANOVA), followed post-hoc by a Duncan's multiple range test at a significance level of 0.05. Significant differences between dominant and subordinate males following aggressive encounters were determined using an independent-samples *t* test ($P < 0.05$).

RESULTS

Brain weights for all spiders ranged from 8.98–9.32 mg (mean: 9.11 ± 0.56 SE). Brain protein/brain weight (%) ranged from 7.2–7.6. An analysis of variance (ANOVA) indicated that there were no differences in mean brain

weights and brain protein values between dominant, subordinate or isolated control spiders ($P < 0.5$). Serotonin was the monoamine found in the highest concentration in the brains of isolated control of *A. hentzi* (Table 1). This was followed in decreasing order by OA, DA, NE, and Epi.

The effects of agonistic interactions between conspecific males on SEG monoamine concentrations at various time intervals following encounter trials are shown in Table 1. Serotonin (5-HT) levels were significantly reduced in spiders losing aggressive encounters (subordinates) for up to 24 h as compared to dominant animals ($t = 9.4$, $P < 0.01$). This difference persisted for at least 24 h, with levels returning to normal after 48 h ($F = 3.36$, $P < 0.05$). In addition, the brains of dominant spiders contained significantly lower levels of 5-HT than those of the isolated controls ($t = 6.8$, $P < 0.05$) for up to 24 h. These changes in 5-HT and OA levels associated with fighting occurred quite rapidly since changes were detected after only 30 min following an encounter.

A similar pattern was found for OA levels which were also significantly reduced in subordinate vs. dominant animals ($t = 6.2$, $P < 0.02$). However, the reduced levels of OA associated with agonistic interactions returned to control levels within 24 hr. With respect to DA, NE, and Epi, no differences were found between spiders exposed to agonistic encounters and controls at any time interval ($P < 0.5$).

DISCUSSION

Although the profile for monoamine concentrations in the SEG of *A. hentzi* (5-HT > OA > DA > NE < Epi) is in general agreement with what little information is available on the neurochemistry of spiders, differences in the NT profiles for spiders from different families have been reported (Florey 1967; Meyer et al. 1984; Meyer 1991) Similar concentrations were reported for NE and DA from the brain of the theraphosid, *Aphonopelma eutylenum* Chamberlin 1918, although no data were presented for 5-HT and OA (Meyer et al. 1984). In contrast, the brain of *Pardosa amentata* (Clerck 1932) contained much higher concentrations of NE (174.9 pmol/mg ± 5.0 SE), a condition most likely associated with the noradrenergic system of the optical

Table 1.—Concentrations of various monoamines (in nmol or pmol/mg protein) in the supraesophageal ganglia (SEG) of *Aphonopelma hentzi* following agonistic interactions between conspecific males. Brain analyses were conducted from tissue extracted from isolated control spiders, as well as from the brains of dominant and subordinate spiders removed 5 min (20 pairs; N = 40) 24 h (n = 20 pairs), and 48 h (n = 20 pairs) after an encounter trial. Data expressed as means; values in parentheses represent (\pm SE). Values followed by asterisks are significantly different than controls: ** ($P < 0.01$); * ($P < 0.05$). See text for details.

Neurotransmitter	Controls	Time after encounter		
		5 min	24 h	48 h
Serotonin (5-HT) (nmol/mg)	89.7 (13.2)			
Subordinate		42.8** (7.6)	51.3** (8.3)	92.2 (12.6)
Dominant		66.5* (9.1)	73.9* (10.4)	86.3 (9.5)
Octopamine (nmol/mg)	56.9 (5.8)			
Subordinate		31.2** (4.9)	54.8 (8.1)	60.1 (10.6)
Dominant		43.7* (7.7)	55.3 (5.2)	57.8 (8.2)
Dopamine (pmol/mg)	22.4 (3.8)			
Subordinate		19.6 (5.1)	23.6 (7.1)	21.9 (5.5)
Dominant		22.2 (7.8)	20.6 (3.5)	24.4 (6.2)
Norepinephrine (pmol/mg)	15.3 (4.7)			
Subordinate		18.1 (5.8)	14.3 (2.9)	17.3 (6.6)
Dominant		16.6 (4.4)	18.5 (3.1)	14.9 (4.1)
Epinephrine (pmol/mg)	0.57 (0.2)			
Subordinate		0.61 (0.1)	0.55 (0.2)	0.58 (0.1)
Dominant		0.52 (0.2)	0.63 (0.3)	0.56 (0.2)

brain centers which are more highly developed in salticids (Meyer & Jehnen 1980). Lycosids and agelenids also contained higher levels of DA and NE as compared to theraphosids, although not as high as salticids (Meyer 1991).

The most pronounced changes in monoamine levels involved 5-HT. They occurred among subordinate males 30 min after fighting, although 5-HT levels were reduced in dominant males as well. Thus, fighting between male spiders resulted in a decrease in SEG serotonin levels. To our knowledge, this is the first demonstration of an association between monoaminergic activity and aggression in spiders. Serotonin has been identified with aggressive behavior in other arthropods as well. Injection of 5-HT into lobsters and crayfish caused them to elevate and flex their tails, which represent behavioral acts associated with the expression of dominance (Yeh et al. 1996).

These observations are interesting since a similar reduction in brain serotonin levels accompanying fighting and territorial defense has been reported for a number of vertebrates. Indeed, it has been well established that a variety of stimuli, including social interactions,

activate endocrine stress mechanisms in vertebrates, which are thought to be mediated by changes in CNS neurotransmitters brought about primarily via activation of monoaminergic systems (Ansell & Bradley 1973; Eichelmann 1987). For example, Summers & Greenberg (1995) showed that 5-HT levels decreased significantly after one h and one day in the brains (diencephalon and non-optic lobe midbrain) of lizards (*Anolis carolinensis*) losing aggressive interactions. Similarly, no changes were detected for NE and DA levels over this time interval. However, subordinate males exhibited significantly lower DA levels after one week than did subordinates after one h. Changes in the serotonergic content and turnover between individuals of different social status were found in the telencephalon and diencephalon of territorial vs. satellite males in the lizard, *Sceloporus jarrovi* (Matter et al. 1998). In addition, the levels of 5-HT in the telencephalon and diencephalon were found to decrease significantly following male-male aggression in rodents (Haney et al. 1990; White et al. 1991) and fish (Winberg et al. 1997).

Immediately following aggressive defense of territories, territorial male lizards (*S. jar-*

rovi) exhibited higher Epi levels as compared to males that did not experience aggressive encounters (Matter et al. 1998). In contrast, no comparable changes in Epi levels in the SEG of *A. hentzi* were observed after fighting.

Octopamine levels also decreased significantly in males of *A. hentzi* exposed to aggressive interactions. This suggests that an activation of the octopaminergic system, in addition to serotonergic activation, follows aggression in spiders. This is not surprising since OA appears to be central in eliciting the overall arousal response of arthropods (Kravitz 1988; Corbet 1991), and elevated OA activity has been shown to accompany stress (Downer & Hiripi 1993; Harris & Woodring 1992), increased locomotor activity (Orchard et al. 1993; Adamo et al. 1995), courtship (Downer & Hiripi 1993), and a wide range of systemic physiological responses including respiration, gastrointestinal peristalsis, cardio-acceleration, Malpighian tubule filtration, glycogenolysis, and pheromone production (Corbet 1991) in insects. It has been further suggested that certain behavior patterns can be triggered by the activation of specific octopaminergic pathways in arthropods, an idea known as the 'orchestration hypothesis' (Sombati & Hoyle 1984). For example, administration of exogenous OA has been shown to trigger diurnal hyperactivity in nocturnal moths (Shimizu & Fukamii 1981). Changes in OA levels in the CNS have been associated with ontogenetic shifts in specific behavioral acts in social insects (Bicker & Menzel 1989; Brandes et al. 1990), and an increase in OA activity was found in the brains of crickets following aggressive interactions between conspecifics (Adamo et al. 1995).

Although most of the research on OA has focused on insects, some previous studies, including the present one, suggest that this monoamine plays an important role in regulating the behavior of other arthropods as well. For example, the tail flip response, an integral behavioral component of the escape response of crayfish, is enhanced by OA (Bicker & Menzel 1989). The injection of OA into freely moving lobsters elicited submissive body postures toward conspecifics (Kravitz 1988). The application of OA caused engorged ticks to detach from their hosts (Mason 1986). With respect to OA, direct comparison

with vertebrates is not possible since OA has not been identified as a NT in this group.

In conclusion, significant changes in brain concentrations of 5-HT and OA result from male-male agonistic encounters in tarantulas. It has been well established that 5-HT is a CNS monoamine involved in the expression of dominance and aggression in vertebrates, and the results of this study suggest that the establishment of social status in spiders causes changes in brain monoamine levels and may play a role in the elicitation of communicative displays as well. Future studies should focus on other species of spiders as well as other arachnids in order to determine if similar changes in monoamine profiles are associated with aggression in these groups.

ACKNOWLEDGMENTS

We are indebted to C. Bradford, R. Khatibi, M. Harvey, and R. B. Suter for commenting on an earlier draft of the manuscript, B. Garmann for consultation on statistical analyses, and J. Bottrell for assistance in the collection of specimens. A University of Tampa Faculty Development Grant and a Delo Foundation Research Grant (DFRG-10-204) to FP made much of this work possible.

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Manuscript received 6 August 2000, revised 2 May 2001.